EURION

European Cluster to
Improve Identification
of Endocrine Disruptors

EURION Stakeholder Workshop

11 December 2020

Summary Report

These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825161 (ATHENA), No. 825762 (EDCMET), No. 825759 (ENDpoiNTs), No. 825753 (ERGO), No. 825100 (FREIA), No. 825489 (GOLIATH), No. 825745 (SCREENED), No. 825712 (OBERON). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

Report of EURION Virtual Stakeholder Workshop

11 December 2020

Summary of the workshop

A EURION virtual stakeholder workshop was held 11 December 2020. It gathered around 60 participants to discuss stakeholder views, needs and expectations related to Endocrine Disrupting Chemicals (EDC) test method development within EURION and international strategies and guidelines. Representatives included regulatory authorities, scientists, industry, civil society, contract research organisations, policymakers as well as other experts working in the field of EDCs. The workshop was hosted by the current EURION cluster coordinators, Joëlle Rüegg (ENDpoiNTs) who welcomed everyone to the workshop, and Henrik Holbech (ERGO) who presented an overview of the cluster, focusing on joint activities.

Pim Leonards (ENDpoiNTs) then summarised the results of a stakeholder survey, to which a total of 73 responses were received from a variety of stakeholders. The survey inquired opinions on the most important criteria to be considered for the development of new tests for endocrine disruptors in EURION, concerning both screening tests (mechanistic) and definitive tests (specific adverse outcomes). Among the survey respondents, the highest importance was assigned to specificity of ED mode of action for the developed test systems (both screening and definitive tests). Interestingly, high-throughput was not among the most important criteria. A majority of the respondents agreed that Adverse Outcome Pathways (AOPs) are useful in the context of developing ED tests, and adding molecular readouts to existing OECD guidelines was also considered important. On the other hand, it was observed that some stakeholders did not agree with these statements. For an overview of the stakeholder survey outcomes see Annex 1.

After the survey results, presentations on stakeholder perspectives were presented. These included contributions from Cristina de Avila (DG ENV) who shared policy insights on the recent EU Chemicals Strategy for Sustainability as well as Niklas Andersson (ECHA) and Andrea Terron (EFSA) who presented a regulatory perspective on the EURION method development. Pia Juul Nielsen (CHEM Trust) highlighted an NGO perspective whereas Helen Tinwell (CEFIC) commented from an industry point of view. Specific questions were then discussed in breakout groups (see below).

The main feedback from the workshop encouraged developing test batteries (not single tests) and reporting results using harmonised templates and with clear guidance for test performance, including uncertainty analysis. It was also highlighted that the *in vitro* endpoints need clear linkage to *in vivo* effects. Stronger involvement of stakeholders was desired as well as frequent communication and dissemination activities. Hence continued stakeholder interaction, surveys and workshops are expected in near future. It was concluded that the discussions have provided important input to the EURION projects and the feedback will be taken into consideration, allowing development of new tests that are applicable and useful for hazard assessment of EDCs.

Summary of the breakout group discussions

Group discussions focused on pre-defined questions. Each group discussed the same questions, but some questions were not discussed due to lack of time in several groups. The summary is based on input from all the six groups as reported in the plenary session, aggregating the answers per question.

Q1: From your perspective, what are the needs and expectations on EDC test method development in EURION?

Several groups highlighted the need to identify new endocrine disruptors and fill the gaps in existing testing batteries with test methods that need to be relevant, robust, reproducible and validated, perhaps even prioritised case by case. It was also emphasised to develop novel assays with human relevance, with specificity for ED mode of action and sensitive enough tests, including windows of susceptibility. The current regulatory required tests are not sufficient to make decisions; tests beyond oestrogen/androgen /steroidogenesis are needed (e.g. other hormonal systems, neurodevelopment, oestrus cycle, thyroid pathway). There is also need for endpoints /parameters and tests to catch metabolic disruptors (insulin axis, energetic metabolism, …) as metabolic outcomes are not well covered in the regulatory field, and metabolically competent *in vitro* assays are needed. For screening assays, it would be useful to have the opportunity for a quick check for EDC effects. It was also hoped that the projects will contribute to easy and fast implementation of test methods and use for regulatory purposes.

Several groups highlighted the need for guidance in the tests and identification of minimum standards: Potential of sensitivity (statistical power), predictivity of mechanistic studies (how predictive are they?), taking more into account fish and mammalian data, and increase understanding on the connecting events. Hence, the methods should be accompanied with proper guidance to address uncertainties and how to interpret data from long term studies where parameters are missing. On the other hand, it was noted that screening batteries may trigger a lot of additional testing, including animal testing because *in vitro* is often not considered enough. In order to address some concerns on relevance of novel *in vitro* methods, there is a need to ensure that *in vitro* endpoints are linked to *in vivo* endpoints. From contract research organisation (CRO) perspective, it was highlighted to know better what type of tests could become available to ease the implementation of the new methods in the laboratories. Modelers, interested in predicting *in vivo* effects from *in vitro*, are eager to have more information on key events (KEs) and key event relationships (KERs). It was noted by one group that the ecotoxicity side is not sufficiently investigated; there should be more focus on tests in invertebrates (not addressed in EURION), and also support for extrapolation from wildlife to human health. It was hoped that ERGO will be able to provide more alternatives linked to *in vivo* endpoints.

Q2: What seems to be the largest obstacles/challenges for the uptake/use of new test methods?

Several groups highlighted gaining confidence of regulators and stakeholder to the new methods as a critical factor. This can be achieved by demonstrating their relevance and validity as well as reproducibility of test results (good negative and positive controls, cytotoxicity testing). Furthermore, confidence can be strengthened through regular stakeholder interaction and communication of these aspects.

Moreover, it was emphasised that it is important to know the limitations of the tests and of the specific methods. Without proper guidance, the interpretation can be quite misleading. More practical implications were also discussed in relation to where to include the guidelines/guidance so that they would be easily available and findable (in the test method or in another document). In case it would be in a separate document, people might less likely read it, so perhaps at least summaries should accompany the test method directly.

A significant challenge is lengthy and complicated legislative and regulatory processes. Furthermore, formal validation at present takes a long time and there is often lack of funding for the validation process and/or OECD work. The level of validation was considered important as well as ranking of the tests to choose which ones to take further into the validation process. Some stakeholders considered OECD approval necessary to have transferable assays to be implemented into the test requirements since the OECD concept of Mutual Acceptance of Data (MAD) is very important for acceptance across the OECD member

countries. Others thought that formal international validation of the screening tests/ OECD TGs are not necessary if looking for a mode of action. It was suggested to fit the testing strategies with clear decision trees. Industry needs legal certainty for the consequences of a test, and therefore it is important to agree on level of validation required to use a method, transferability, accessibility of technology and costs. Post market re-evaluation of methods was encouraged as well.

Additionally, one chemical can produce effects on different endpoints – how to address that in the best possible way? A need for systematic and predefined data search strategy was also emphasised. When modelling starts taking on, it will require big data sets and proper infrastructure. Some concerns were expressed on a large number of various *in vitro* assays for one modality which can lead to picking up compounds that may not have effects in *in vivo*. Consideration of concentrations used *in vitro* was also highlighted, as they should be relevant to effects seen *in vivo*. Furthermore, it was suggested not to focus only on the "usual suspect" substances but investigate specificity and sensitivity of methods with weak and negative substances (test a lot of chemicals during development/validation).

Q3: What type of validation is needed for *in vitro* screening assays and *in silico* methods?

Validation implies that other people can use an assay and have the same results, but the need for validation also depends on the maturity of the test. More concern was expressed about false negatives than false positives, as high sensitivity is a key to protect "consumers' risk" and public health.

In silico methods should be validated as well, and the validation procedure could include inter-expert comparisons, but this also depends on the methods. Need of GLP and reproducibility of the method to be validated was also raised as well as acceptance across countries. The OECD process delivers good quality but the process in general takes time. It was discussed whether it is always necessary to have an OECD TG or would good assays, fit-for-purpose, be sufficient even if not validated at the OECD level. Robustness criteria should be developed in any case in the context of the specific test method.

For validation purposes, it would be useful to make sure that what is published is of good quality to be used in a regulatory context. Scientists need to be aware of what is needed for a regulatory assessment. Hence, peer-review of scientific articles could ask more questions to exactly understand how data have been produced (and one can use already existing guidelines/tools for such data reporting).

Q4: What type of specific endpoints should be added to existing OECD guidelines?

Some stakeholders recommended that the existing guidelines should be updated (almost 20 years old). There is a need to compare responses between different species and to address *in vitro* to *in vivo* extrapolation as well as Physiologically Based PharmacoKinetic (PBPK) models. There should also be development of methods to include sensitive endpoints. A better definition of windows of susceptibility should be agreed upon. Behaviour is a very sensitive endpoint, and it is not certain whether it can be covered by biomarkers. Some endpoints are difficult to interpret (importance to do these measurements in control animals to have historical data to refer to). Regarding e.g. fish, different species should be used.

It was stated that the androgen area is quite well covered and has adequate testing methods, but some issues still need to be addressed with regard to e.g. oestrogenic chemicals. In terms of the thyroid hormone system, there are no validated thyroid assays which can identify downstream effects on the brain (not the thyroid gland itself). Some stakeholders expressed that it is encouraging that there are methods to be developed in EURION for metabolic pathways and to study how EDs can affect metabolism. It was also brought up that intermediate key events could be investigated as part of the method development.

Q5: What is your opinion on the addition of molecular readouts to existing OECD guidelines?

Several groups noted that molecular readouts are useful for the development of AOPs and it could be considered adding them to existing guidelines if practically implementable (including considerations of cost and availability). For risk assessments having molecular readouts in the context of AOPs will help (can be used as part of Weight of Evidence, WoE), but more work is needed in the development of these studies, also to overcome potential reproducibility concerns. One alternative is to use molecular information for screening tests rather than for amendments of existing test guidelines. Some stakeholders considered molecular readouts a rather mechanistic approach whereas some wanted to know how easy it would be to add such molecular readouts to studies and how to interpret the data generated. It was also suggested that a pattern recognition model combined with molecular descriptors, using Bayesian models, could be used together to better inform on the endpoint. More contributors in this area would be welcome as it is a promising field and predictions work well in some cases. A challenge is that there is no clear consensus on how to interpret these readouts, but EURION can contribute to overcoming this challenge.

Q6: What is your view on the usefulness of AOPs in the context of developing ED tests?

Several groups considered AOPs/AOP networks as a valuable tool, though not necessarily an end in itself. If a regulatory assessment is based on an AOP requirement, it could become problematic if that would imply that a full AOP network needs to be established before a compound can be identified as an ED. AOPs were considered useful in helping to decide what kind of test methods are useful or needed. One *in vitro* assay will not address all the key events. It was considered important to link modes of action to AOPs and to develop more AOPs (including more endocrinology-related) useful for regulatory purposes. It was also brought up that many AOPs probably convert at some very crucial KEs.

On the other hand, some expressed concerns about the linearity and simplistic framework, as well as the amount of evidence needed to demonstrate essentiality (balance between enough evidence *vs* time spent). All the details might not be needed for regulatory purposes. In addition, there is difficulty linking some general effects (e.g. ROS production) to ED-related effects. Another type of challenge is that the AOP-Wiki is not enough populated, and there is no broad public discussion (would be helpful to increase AOP acceptance). There seems to be reluctance to include data in the Wiki before they are published, and this obviously takes time. In the long-term, it may be difficult to realise this potential without a good software to support the links to the existing AOPs.

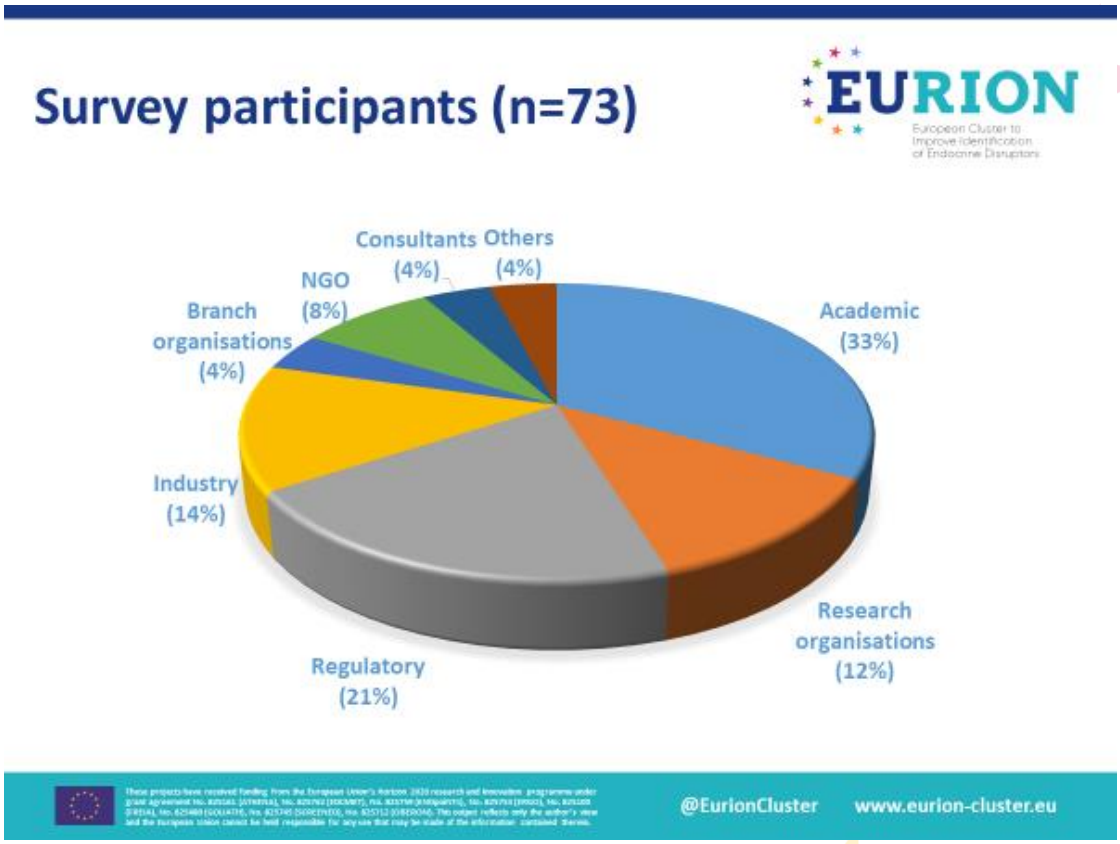
Q7: What should be the benefit of grouping and read-across in ED assessment [e.g. cumulative risk assessment]?

Being able to group chemicals with the same mode of action is very useful as we will not be able to test all substances. It will lead to less tests, less animal use and less time required for assessment. At the same time, it was noted that it is not enough on its own nor straight forward and should be clearly evaluated for the assessment. The “burden of proving otherwise” could be put to substance producers if convinced that a single substance does not have similar effects as the other substances in the assessment group.

Acknowledgements to the chairs and rapporteurs of the group discussions: *Majorie van Duursen and Sharon Munn; Pim Leonards and Helle Lyngborg; Juliette Legler and Elise Grignard; Andreas Kortenkamp and Asma Baig; Robert Barouki and Marie Hauduroy; Anna-Liisa Levonen and Jenni Küblbeck.*

Annex 1: A summary of the stakeholder survey results (presented below).

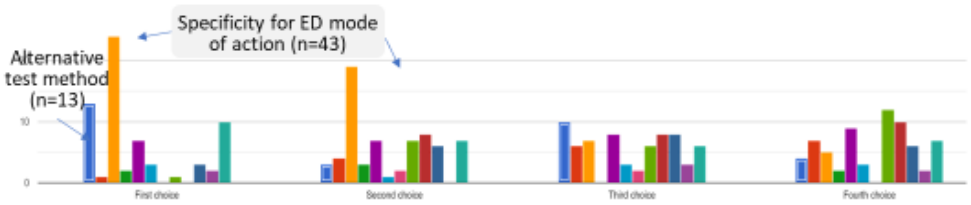
The survey inquired opinions on the most important criteria to be considered for the development of new tests for endocrine disruptors (EDs) in EURION.



Screening tests informing mainly about mechanistic effects

- Alternative test method (*in vitro*, *in chemico/silico*, whole organism embryos)
- High throughput
- Specificity for ED mode of action
- Whole organism
- OECD accepted
- Simplicity
- Low cost
- High sensitivity
- High specificity
- Adverse outcome pathway-based
- Short duration
- Suitable for screening

Rank the six most important ones.
First choice: Highest importance.

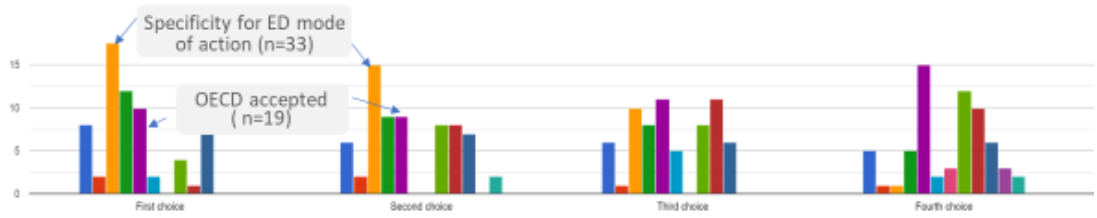


These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825161 (ATHENA), No. 825762 (EDCMET), FKG. 825759 (ENDPHYS), No. 825753 (ENDOS), No. 825160 (EDTA), No. 825469 (GOLIATH), FKG. 825745 (SCREENED), FKG. 825712 (OBERON). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

Definitive tests informing about mechanistic and adverse effects

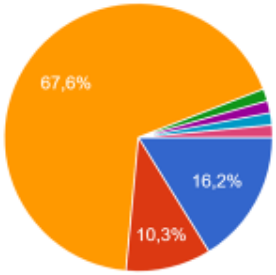
- Alternative test method (*in vitro*, *in chemico/silico*, whole organism embryos)
- High throughput
- Specificity for ED mode of action
- Whole organism
- OECD accepted
- Simplicity
- Low cost
- High sensitivity
- High specificity
- Adverse outcome pathway-based
- Short duration
- Suitable for screening

Rank the six most important ones.
First choice: Highest importance.



These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825161 (ATHENA), No. 825762 (EDCMET), FKG. 825759 (ENDPHYS), No. 825753 (ENDOS), No. 825160 (EDTA), No. 825469 (GOLIATH), FKG. 825745 (SCREENED), FKG. 825712 (OBERON). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

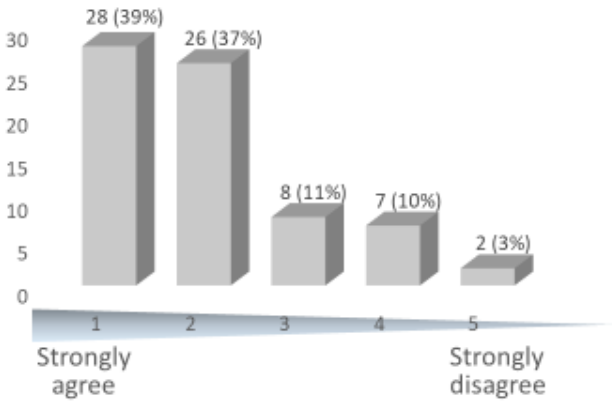
Method development within EURION should mainly focus on



- screening tests
- definitive tests
- both
- Both, and linking screening and definitive tests via AOP networks
- Would like to see focus on advancing screening test that could gain acceptance as definitive test
- Robust and validated methods (ring-trail tested)
- Focus should be directed towards development of "alternative" non-animal test methods that provide information requirements on Data from new approach methodologies (in vitro, in silico, omics, etc.) thoughtfully and creatively combined and applied to an AOP framework may provide sufficient information needed for risk assessment.

These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825161 (ATHENA), No. 825762 (EDCMET), No. 825759 (ENDOMET), No. 825753 (ERGO), No. 825100 (FRIDA), No. 825489 (GOLIATH), No. 825345 (GREENED), No. 825712 (OBERON). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

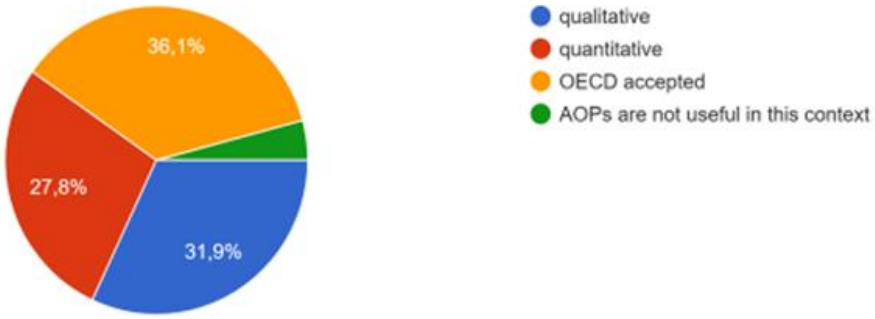
Adverse Outcome Pathways (AOPs) are useful in the context of developing ED tests



These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825161 (ATHENA), No. 825762 (EDCMET), No. 825759 (ENDOMET), No. 825753 (ERGO), No. 825100 (FRIDA), No. 825489 (GOLIATH), No. 825345 (GREENED), No. 825712 (OBERON). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

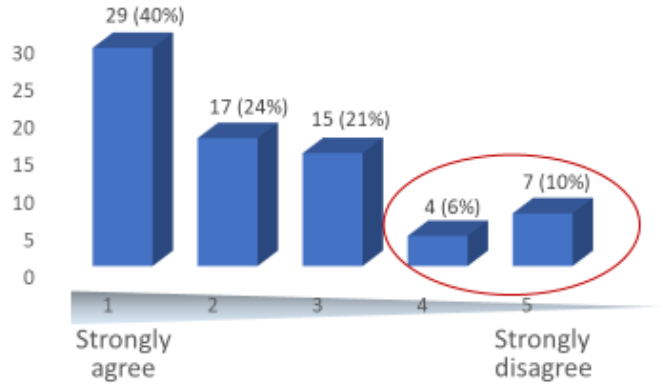
@EurionCluster www.eurion-cluster.eu

In order to base a testing method on an AOP, the AOP has to be...



These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 825161 (CERTISTA), no. 825762 (EDCMET), no. 825759 (ENDOMETH), no. 825753 (ERGO), no. 825100 (FATE-HUB), no. 825489 (GOLIATH), no. 825745 (SOLVENTO), no. 825712 (OBERON). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

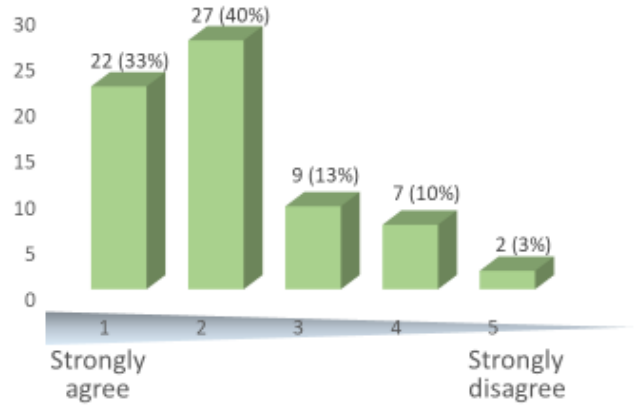
Adding molecular readouts (e.g. transcriptional/metabolomic changes) to existing OECD guidelines is valuable



These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 825161 (CERTISTA), no. 825762 (EDCMET), no. 825759 (ENDOMETH), no. 825753 (ERGO), no. 825100 (FATE-HUB), no. 825489 (GOLIATH), no. 825745 (SOLVENTO), no. 825712 (OBERON). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

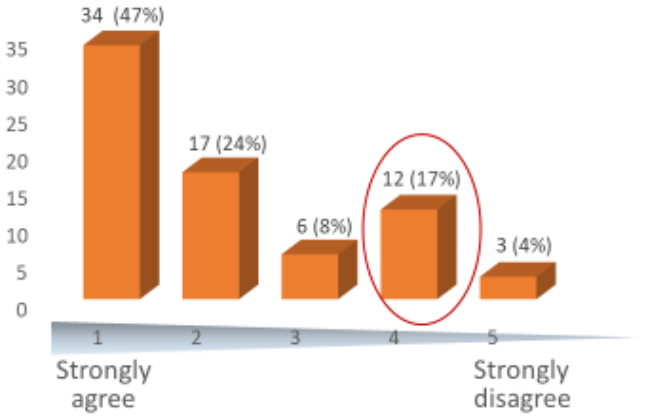
@EurionCluster www.eurion-cluster.eu

Read-across between vertebrate classes (e.g. mammals and amphibians) for assessing ED is valuable



These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825161 (ATHENA), No. 825762 (EDCMET), No. 825759 (ENDOMET), No. 825753 (ENDOC), No. 825100 (FREIA), No. 825489 (GOLIATH), No. 825345 (SCREENED), No. 825712 (OBERON). The output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

Grouping of substances and read-across (predicting properties from one chemical to other similar chemicals) for assessing ED is valuable



These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825161 (ATHENA), No. 825762 (EDCMET), No. 825759 (ENDOMET), No. 825753 (ENDOC), No. 825100 (FREIA), No. 825489 (GOLIATH), No. 825345 (SCREENED), No. 825712 (OBERON). The output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

@EurionCluster www.eurion-cluster.eu

Contact

EURION Coordination 1 April 2020 - 30 June 2021

Joëlle Rüegg (ENDpoiNTS), email: joelle.ruegg@ebc.uu.se

Henrik Holbech (ERGO), email: hol@biology.sdu.dk

Author of the report:

Elina Drakvik (ENDpoiNTs)

Design:

AquaTT